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Potential Role of Microorganisms Isolated from Periodontal Lesions in the Pathogenesis of Inflammatory Bowel Disease

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A total of 20 patients with inflammatory bowel disease (IBD) (Crohn's disease, ulcerative colitis) were evaluated with regard to the role of infectious agents and host response. Patients were selected based upon oral manifestations of their disease, 10 with periodontal disease and 10 without. Microbiologic studies of the periodontal flora of IBD-affected patients revealed a unique microflora composed predominantly of small, motile, gram-negative rods, which were most consistent with the genus *Wolinella*. Further studies of the host response of these patients revealed a serum-mediated defect in neutrophil chemotaxis in all 10 patients with periodontal disease. Neutrophil phagocytosis was normal. In vitro studies of neutrophil function in response to *Wolinella* extracts and culture supernatants revealed inhibition of neutrophil chemotaxis in a dose-response fashion. The organism was chemokinetic for neutrophils but not chemotactic. The data suggest that unusual microorganisms colonizing the oral cavity of IBD patients potentially play a role in the pathogenesis of the disease as infectious agents or modifiers of the host response or both.

Inflammatory bowel diseases (IBDs) have become a significant public health problem because of the increase in the incidence of IBD, particularly Crohn's disease, in the U.S. population (45). Ulcerative colitis and Crohn's disease are chronic intestinal disorders of unknown etiology. Many different insults may acutely inflame the intestinal mucosa, but the pathogenesis of the chronic inflammatory lesion in IBD may be entirely unrelated to the nature of the initial insult. Despite recent interest in transmissible agents, cytopathic effects in cell lines, and the role of cell-wall-deficient pseudomonads (L forms), there is still no evidence for an infective etiology for IBD (2, 16, 20, 25, 34, 40, 42, 53).

Ulcerative colitis is a mucosal disease of the colon that does not involve the small intestine. It begins at the rectum and extends proximally. Histologic evaluation of colonic tissue reveals all the features of a classic Arthus reaction, including the acute inflammatory infiltrate of neutrophils, eosinophils, and plasma cells associated with edema, vascular dilation, and diapedesis. In contrast, Crohn's disease may affect any part of the gastrointestinal tract and may have extraintestinal manifestations as well. Crohn's disease is characterized by a transmural inflammation of lymphocytes, macrophages, and plasma cells, and in 70% of the cases, granulomas are found. Extraintestinal manifestations include mouth ulcers, severe periodontal disease, and involvement of heart and lungs, skin, joints, liver, and uveal tracts (3, 4, 14, 15, 22, 35, 36).

Previous studies (1, 27, 41) have demonstrated that patients with IBD exhibit depressed neutrophil chemotaxis in vitro. Concomitantly, severe periodontal disease has been noted as a characteristic feature in certain IBD patients (12, 27, 41). The neutrophil abnormalities observed in patients with IBD are also observed in patients with certain types of periodontal diseases, most notably localized juvenile periodontitis (8, 9, 28, 49). This is not surprising, since the neutrophil has been shown to be an important cell in host resistance to periodontal breakdown (39). Since periodontitis is an infectious disease, we investigated the periodontal microflora in IBD patients and found them to be unique and uncharacteristic of other periodontal diseases. The periodontal microflora of IBD patients is composed almost completely of small, motile, anaerobic, gram-negative rods.

Our studies focused on the periodontal manifestations of IBD for three major reasons: (i) the similarities in the pathogenesis of the diseases, (ii) the simultaneous occurrence of the diseases, and (iii) the unusual microflora found associated with periodontal disease in IBD patients.

In this paper, we report the isolation of unusual microorganisms from the periodontal lesions of IBD patients. We also report the occurrence of serum inhibitors of neutrophil chemotaxis in IBD patients, which may be related to a factor(s) elaborated by the microflora of these patients.

MATERIALS AND METHODS

Subjects. Patients included in the study were from Emory University School of Dentistry, Emory Periodontal Research Center, Emory Clinic, and affiliated hospitals. Informed consent was obtained from all subjects. A complete medical history and a battery of clinical laboratory tests, including SMA-22, prothrombin time, and urinalysis, were completed on all patients. Health questionnaires were administered that elicited information regarding tobacco and alcohol use, drugs and medications, illnesses and blood loss; for females, additional information regarding birth control medication, menstruation, and pregnancy was gathered. Subjects were not included if they were pregnant or if they were acutely ill, smoked more than 10 cigarettes daily, consumed more than 2 oz of alcohol (~60 ml) daily, or were taking any medication within 24 h before samples were taken, with the exception of anti-inflammatory agents, which were recorded. Patients with a positive history of antibiotic therapy within 6 weeks of the sampling date were not included for study.

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Three categories of patients plus one of controls were studied. Category 1 included 10 patients with IBD who had a confirmed diagnosis of ulcerative colitis or Crohn's disease and significant periodontal disease. IBD patients were defined as those exhibiting a combination of symptoms, including diarrhea, abdominal pain, bleeding, weight loss, perianal disease, and arthritis. Steroid usage history of these patients was recorded. Category 2 consisted of 10 IBD patients without any periodontal involvement. Category 3 had 8 patients with adult periodontitis. Adult periodontitis controls were age and sex matched and were matched to the type and severity of periodontal disease in the IBD group. Control subjects consisted of healthy individuals from the laboratory or student populations who had no radiographic evidence of alveolar bone loss or no clinical evidence of periodontal disease other than mild gingivitis.

Evaluation of periodontal status. The periodontal status of patients and controls was determined by a standard set of clinical, radiographic, biochemical, and microscopic measurements used at the Emory Periodontal Research Center. The complete periodontal examination includes assessment of gingival erythema, edema, suppuration, bleeding upon probing, and pain upon probing. Additionally, data collection includes enumerating the morphotypes of microorganisms in eight periodontal sites by dark-field microscopy and measuring crevicular fluid (CF) prostaglandin E_2 (PGE_2) levels for each tooth at the mesiofacial line angle. Ramfjord attachment levels (3) were measured with a controlled-force probe set to deliver a constant probing force of 28 g. Radiographic assessment of bone loss was made with periapical films taken by the long-cone parallel technique, and bone loss was quantified by measuring the distances from the cemento-enamel junction to the crest of bone on the mesial and distal points of each tooth (5). AAP classification of disease severity was used to match patients (17).

Dark-field microscopy. A previously described standardized technique (18, 31) was used for enumerating and identifying various morphotypes of microorganisms in samples from periodontal pockets by dark-field microscopy. A syringe technique was used for collecting the sample in heparinized saline from the periodontal pocket. The syringe technique provides a standardized subgingival plaque sample from the base of the pocket, which probably reflects the composition of loosely rather than tightly adhering plaque on the tooth surface.

Isolation and identification of microorganisms. (i) Sample collection. The gingival and supragingival tooth margins were carefully cleansed of saliva, debris, and plaque with sterile gauze, and specimens were collected by the syringe technique described above. A 2-μl sample was then immediately placed in 7 ml of prereduced Lombard-Dowell medium (11) and taken to the laboratory for culture.

(ii) Sample processing. The sample material was dispersed with a vortex mixer for 60 s, serially diluted in 10-fold steps in the same medium, and plated on prereduced Centers for Disease Control (CDC) anaerobe blood agar (11) and various selective media, including malachite green bacitracin agar (32), TBBP medium (33), CVE medium (54), phenethyl alcohol blood agar (11), kanamycin-vancomycin blood agar (11), modified kanamycin-vancomycin laked blood agar (47) with CDC anaerobe blood agar as the base, Columbia colistin nalidixic acid blood agar (52), and CDC anaerobe blood agar containing 5 μg of clindamycin per ml. In addition, half of each sample was passed through a sterile membrane filter (0.45-μm pore size) and aliquots of the filtrate were used to inoculate CDC anaerobe blood agar, enriched thioglycolate broth, and hippurate-formate-fumarate broth (26). After inoculation, all media were incubated in an anaerobic glove box containing 5% CO_2, 10% H_2, and 85% N_2. Isolates were tested with a battery of standardized tests used in the CDC Anaerobe Reference Laboratory and identified according to current recommendations published in the *International Journal of Systematic Bacteriology* and elsewhere (13, 23, 46).

Determination of CF-PGE_2. CF was collected, and PGE_2 was determined in each sample as described previously (38). Briefly, CF was collected on periopaper strips, and the volume was determined with a periotron (Harco). PGE_2 concentration was determined after elution of CF from periopaper strips by centrifugal filtration and radioimmunoassay (37).

Isolation of neutrophils. Neutrophils were isolated as previously described (50). Neutrophils were separated from heparinized venous blood by Ficoll-Hypaque centrifugation, washed twice in phosphate-buffered saline (PBS) (pH 7.2), and suspended in appropriate assay medium. Whenever possible, assays were performed simultaneously on samples from the same batch of cells.

Chemotaxis. The chemotaxis assay, as it is performed in our laboratory, has been described in detail elsewhere (50). Neutrophils were suspended in an assay medium consisting of Gey's balanced salt solution (GBSS) supplemented with 2% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) at a concentration of 2.5×10^6 cells per ml. The cell suspension was placed in the upper compartment of a modified Boyden chamber separated by a micropore filter (5-μm pore size; Sartorius Membranfilter GmbH, Gottingen, Federal Republic of Germany). The lower compartment contained the synthetic chemotactic peptide *N*-formylmethionylleucylphenylalanine (FMLP) (2×10^{-8} M) or endotoxin-activated serum (EAS) (at a predetermined optimal concentration). Chemotaxis was evaluated by counting the number of neutrophils that accumulated on the distal surface of the filter after a 60-min incubation. Ten high-power fields (×400) were counted for each triplicate filter. Assays were performed on at least two separate days and, for most patients, on three or more days. Statistical differences between the patient and the control were determined by analysis of variance.

Phagocytosis. The phagocytosis assay used is an adaptation of the method of Van Furth et al. (51). Isolated neutrophils were suspended in sterile GBSS with 0.1% gelatin at a concentration of 10^7 cells per ml. The indicator organism, *Staphylococcus aureus* (protein A negative), was grown on slants of brain heart infusion agar (BBL Microbiology Systems, Cockeysville, Md.) and washed in PBS, and the concentration was adjusted to an optical density of 0.6 at 540 nm. The organisms were centrifuged at 10,000 × *g* and suspended in 5 ml of a 1:10 dilution of pooled human serum for opsonization for 30 min at 37°C. After two washes in PBS, the organisms were suspended in GBSS at 4×10^8 cells per ml. Optimal concentration and ratios of bacteria and neutrophils were predetermined, and no difference was noted between the patient and the control. The reaction mixture consisted of 0.5 ml of neutrophil suspension and 0.5 ml of preopsonized *S. aureus* suspension in Falcon 2003 tubes (Becton Dickinson Labware, Oxnard, Calif.). The mixture was gently inverted for 30 min at 37°C on an Ames aliquot tilt table. The reaction was stopped by the addition of 3 ml of cold GBSS, and the mixture was centrifuged at 400 × *g* for 5 min. The pellet was suspended in 0.2 ml of a 20-U/ml solution of lysostaphin (Sigma) in PBS to lyse extracellular

bacteria, including those attached to the surface of the neutrophils. Aliquots (40 μl) were then placed in raised drops on clean glass slides and incubated for 15 min at 37°C in an atmosphere of 5% CO_2 in air. The slides were washed with PBS and dried and then Gram stained. Phagocytosis was quantified by counting the number of *S. aureus* cells in each of 30 to 50 bacteria-containing neutrophils per slide, as viewed under oil immersion at ×1,250 magnification. In addition, the percentage of 100 neutrophils that contained bacteria was determined. Assays were performed in duplicate on at least two separate days. Statistical differences between the patient and the control were determined by analysis of variance.

Bacterial inhibition of neutrophil function. Inhibition of neutrophil chemotaxis and phagocytosis by both sonic extracts and culture supernatants of periodontal microorganisms (7, 30, 48) suggests that these products may play a role in the alteration of neutrophil function in vitro. Washed neutrophils (10^7/ml) were incubated with 0.01, 0.1, 1.0, 10.0, and 100 μg of a sonicate preparation of the IBD isolates, stock strains of *Wolinella* and *Campylobacter*, or control medium for 15 min in a shaking water bath at 37°C and then washed twice in PBS. The washed neutrophils were suspended in GBSS and assayed for chemotaxis and phagocytosis. Statistical differences between the experimental and control samples were determined by analysis of variance.

The chemotactic activity of the IBD bacterial preparation and stock strains was assessed by placing the sonicate in the lower compartment of the Boyden chamber in the chemotaxis assay. No other chemotactic agent was included in the system for these experiments. Concentrations of sonicate ranging from 0.01 to 100 μg/ml were used to obtain a dose-response curve.

Serum inhibition of neutrophil function. The presence of inhibitors of chemotaxis and phagocytosis in the serum of IBD patients was evaluated by placing patient or control serum in the assay medium at a concentration of 5%. Care was taken not to create a serum gradient in the Boyden chamber assay by placing equal concentrations of serum in both compartments of the Boyden chamber.

RESULTS

Dark-field microscopy. Dark-field microscopy examination of the oral microflora of IBD patients with and without periodontal disease revealed that the dominant organism (>99%) was a very small, motile rod. The characteristic spirochetal and filamentous flora seen in adult periodontitis is absent in the IBD patients. Controls exhibited normal flora.

Isolation and identification of the IBD microflora. The predominant isolate from IBD patients was a nonsporeforming gram-negative rod with unipolar flagella. Some isolates of this organism(s) could be separated from larger organisms by filtration through a 0.45-μm-pore-size filter. Preliminary tests revealed that the characteristics of the isolates were consistent with the genera *Wolinella* and *Campylobacter*. Reference strains of these organisms, including *Campylobacter concisus*, *Wolinella curva*, *Wolinella recta*, and *Wolinella succinogenes*, were then used for comparison. Ten of the IBD isolates from the ten IBD patients with periodontal disease were characterized and are reported here. The morphologic features of the IBD isolates were compared with those of the reference strains. Relevant biochemical and other morphologic characteristics are summarized in Table 1. These data demonstrate that the isolates are distinct from the species represented but are most closely related to the genus *Wolinella*. It is of interest that in the periodontal-disease-free IBD patients the bacterial load in the gingival sulcus was greatly reduced from that of the IBD group with periodontal disease. However, the flora was similar in composition in all 10 periodontal-disease-free IBD patients and comparable to that of IBD patients with periodontal disease.

Clinical evaluation. Clinical evaluation of the periodontal status of IBD patients was performed as described previously (38). These data were compared with similar measurements made on patients with adult periodontitis and on normal, healthy controls. As can be seen (Table 2), the clinical picture of the periodontal health of the IBD patient is one of moderate to severe periodontitis with extreme inflammation.

Steroid usage. Of the IBD patients identified for this study, half reported a history of steroid therapy within the last year or were currently taking steroids. The other half either had never taken steroids or had not taken steroids within the past 2 years. There were no apparent associations among microbial composition, neutrophil function, and steroid usage in these patients.

CF-PGE_2 concentration. Studies in our laboratory have revealed a strong correlation between the amount of periodontal destruction and the concentration of PGE_2 found in the gingival CF. We measured the CF-PGE_2 levels in samples from eight IBD patients with periodontal disease and age-, sex-, and periodontal disease-matched adult periodontitis patients. Since it is reasonable to assume that PGE_2 levels reflect the degree of inflammation (37), it is of note that the PGE_2 levels of the IBD patients were four times those of the control group (210.9 ± 44.6 [standard deviation] versus 65.9 ± 14.5; $n = 8$).

Neutrophil chemotaxis in IBD. Neutrophil chemotaxis to endotoxin-activated EAS and FMLP was evaluated in both a serum-free system and in the presence of 5% serum. The IBD group was subdivided into patients with ulcerative colitis and those with Crohn's disease. IBD patients were further divided into two groups, one with periodontal disease and one without. In a serum-free system, the chemotactic response of neutrophils from patients with Crohn's disease who had periodontal disease was not different from that of the normal control population. However, patients with ulcerative colitis exhibited reduced chemotactic response to both agents tested (Table 3). Of the IBD patients without periodontal disease, there were mixed results, with two of six Crohn's disease patients and two of four ulcerative colitis patients exhibiting defects (data not shown).

In the presence of 5% patient serum (Table 4), the chemotactic response of normal neutrophils was significantly inhibited by all serum from patients with IBD and periodontal disease. The chemotactic response of normal neutrophils was not affected by serum from normal donors. Mixed results were obtained from periodontal-disease-free IBD patients, as the sera of two of six patients with Crohn's disease and two of four patients with ulcerative colitis inhibited chemotaxis (data not shown).

Neutrophil phagocytosis in IBD. The phagocytic ability of neutrophils from IBD patients was compared with that of normal controls, with opsonized *S. aureus* as the indicator organism. Phagocytosis was quantified as the bacteria per neutrophil and as the number of neutrophils containing bacteria. In both instances, no difference was noted between IBD patients and controls (data not shown).

TABLE 1. Comparison of relevant characteristics of the IBD isolates with those of phenotypically similar motile, gram-negative microorganisms

Species and strain no.	Rod morphology	Motility type[a]	Relationship to oxygen[b]	Effect of CO_2 on growth[c]	Colony size on blood agar (diam in mm)	Pigmentation on blood agar	Greening of blood agar around colonies	Oxidase[d]	Flagella[d,e]
C. concisus ATCC 33237	Medium, curved	RU	MA	1	<1	None	−	+	SPF
W. curva ATCC 35224	Tiny, curved	T	MA	0	1–3	None	−	+	SPF
W. recta ATCC 33238	Medium, straight	T	AN	2	1–3	None	−	−	SPF
W. succinogenes ATCC 29543	Small, straight	T	AN	R	<1	None	+	−	SPF
IBD isolates									
A-6922	Small, straight	RU	AN	0	<1	Tan	−	−	SPF
A-6679	Tiny, straight	RU	AN	0	<1	Tan	−	+	SPF
85-0797-2	Small, straight	T	AN	0	<1	None	−	−	N
85-0797-4	Medium, straight	T	AN	0	1	None	−	−	SPF
85-0797-5	Tiny, straight	T	AN	0	1	None	−	−	N
85-0797-6	Medium, straight	T	AN	2	<1	None	−	−	N
85-0797-8	Small, curved	T	MA	2	<1	None	−	+	SPF
85-0808-2	Small, straight	T	AN	0	<1	None	−	−	N
85-0808-6	Small, curved	T	MA	2	<1	None	−	+	SPF
86-0014-2	Small, straight	T	MA	2	<1	None		+	SPF

[a] RU, Rapid, unidirectional; T, tumbling.
[b] MA, Microaerotolerant anaerobe; AN, obligate anaerobe.
[c] 0, Little or no stimulation; 1, moderate stimulation; 2, marked stimulation; R, required.
[d] All isolates were indole and catalese negative.
[e] SPF, Single polar flagellum; N, no flagella.

Bacterial inhibition of neutrophil function. The isolation of an organism that constitutes more than 99% of the periodontal flora of IBD patients and the concomitant serum-mediated neutrophil chemotaxis defect suggest that these organisms may be exerting a direct effect on neutrophil function. This hypothesis was tested by incubation of neutrophils with sonic extracts of the bacterial isolate before the functional assay.

(i) Chemotaxis. The inhibitory capacity of increasing concentrations of *Wolinella* sonic extract on the chemotactic response of normal neutrophils was evaluated with both EAS and FMLP. Neutrophil viability was not affected at any concentration of bacterial extract tested, as evaluated by trypan blue dye exclusion. There was a clear dose response of inhibition with increasing concentrations of IBD isolate extract, reaching a maximum of 70% inhibition at the highest doses. This was in marked contrast to laboratory stock strains of *W. recta* and *C. concisus*, which did not inhibit function (Fig. 1).

(ii) Phagocytosis. The inhibitory capacity of increasing concentrations of *Wolinella* extract on the phagocytic response of normal neutrophils was tested with *S. aureus* as the indicator organism. No differences were noted when neutrophils were pretreated with *Wolinella* extract or when

TABLE 2. Clinical evaluation of peridontia of IBD patients[a]

Patients	% Patients with:		Mean attachment loss (mm) ± SEM
	Redness	Bleeding on probing	
IBD with PD	100[b]	37.5[b]	1.1 ± 0.6[b]
AP	47.6[b]	25.9[b]	3.2 ± 2.0[b]
IBD with no PD	1.1	0.4	0.2
Control	1.5	0.6	0.2

[a] There were 10 individuals in each group. PD, Periodontal disease; AP, adult periodontitis. Redness and bleeding were recorded as dichotomous variables. Comparison of IBD-PD and AP groups revealed no statistically significant differences in attachment loss between groups. Both groups were classified as AAP type III cases.
[b] Significantly different from control at $P < 0.05$ as determined by analysis of variance.

TABLE 3. Neutrophil chemotaxis in IBD evaluated in the Boyden chamber assay in a serum-free system[a]

PD patient with either Crohn's disease or ulcerative colitis	Mean chemotactic response as % of control ± SEM	
	EAS	FMLP
Crohn's disease		
1	102.0 ± 10.1	88.5 ± 8.7
2	100.0 ± 9.7	85.0 ± 8.6
3	100.0 ± 10.0	101.0 ± 11.0
4	118.0 ± 8.7[b]	121.0 ± 9.2[b]
5	100.0 ± 6.7	126.0 ± 5.7[b]
6	73.7 ± 5.2	71.7 ± 6.1[b]
Ulcerative colitis		
7	67.0 ± 6.1[b]	52.7 ± 5.2[b]
8	52.3 ± 4.7[b]	58.3 ± 5.3[b]
9	50.2 ± 3.6[b]	52.7 ± 4.3[b]
10	48.2 ± 7.1[b]	49.6 ± 5.7[b]

[a] For each patient, age- and sex-matched controls were used for each test. PD, Periodontal disease. No difference in neutrophil response was demonstrable between patients with Crohn's disease and the control patients. However, all ulcerative colitis patients with periodontal disease exhibited a significant ($P < 0.05$) reduction in neutrophil chemotaxis.
[b] Statistically significant at $P < 0.05$.

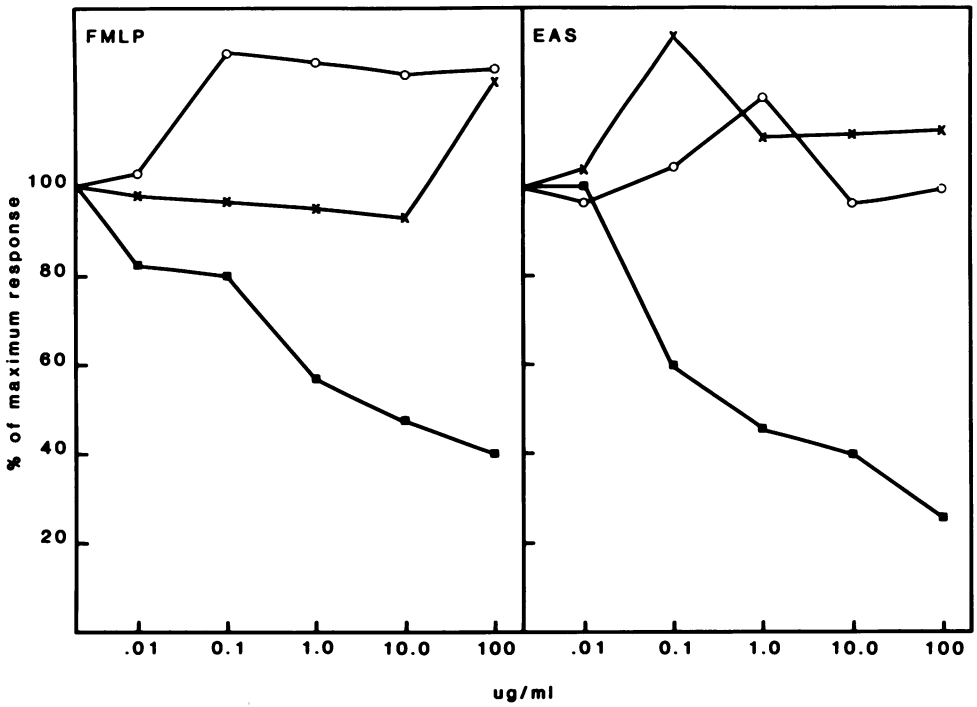

FIG. 1. Inhibition of neutrophil chemotaxis by IBD isolates. The chemotactic response of normal neutrophils to the chemotactic factors FMLP and EAS is represented as the percentage of the maximal response obtained under the conditions of the assay. The concentration of bacterial extract is represented on the x axis in micrograms per milliliter of lyophilized extract. The dose-response curves clearly indicate a concentration-dependent inhibition of neutrophil chemotaxis by the extract of the *Wolinella* isolate from IBD (■). There was no neutrophil toxicity detected as evaluated by trypan blue dye exclusion. Two reference strains of closely associated organisms, *W. recta* (○) and *C. concisus* (×), were also tested and demonstrated no activity. Statistical differences were determined by analysis of variance. For both FMLP and EAS, there was a statistically significant inhibition of neutrophil chemotaxis at an IBD sonicate concentration of 1 μg/ml.

the experiment was performed in the presence of *Wolinella* extract (data not shown).

Elaboration of chemotactic factor by *Wolinella* spp. To determine whether a chemotactic factor(s) was being elaborated by the *Wolinella* isolate from IBD, a checkerboard experiment was performed. The checkerboard experiment was based on the assumption that there will be a greater response in the Boyden chamber assay of neutrophils in a chemotactic gradient than in the absence of a chemotactic gradient. Data from this experiment indicate that the organism has only chemokinetic properties and is not chemotactic for neutrophils. Reference strains of *Wolinella* and *Campylobacter* were chemokinetic but not chemotactic as well.

DISCUSSION

We approached two aspects of the pathogenesis of IBD: (i) the problem of infectious agents and (ii) the question of phagocytic cell response in the IBD patient. The selection of our patient population was based on the observation that a subpopulation of IBD patients exhibits severe gingival and periodontal disease as an extraintestinal manifestation of their disease.

The periodontal microflora of these patients is unlike that of patients exhibiting other defined forms of periodontal diseases. By dark-field microscopy, the predominant morphotype found was a tiny, gram-negative, motile, unipolar flagellated organism. In contrast, in other periodontal diseases many spirochetes, filamentous, fusiform, and other larger motile organisms are characteristic (31). Cultural studies of oral isolates revealed that these tiny organisms are consistent with *Wolinella* species. Much of the data regarding infectious agents in IBD are based upon transmissibility of the disease after filtration of Crohn's disease tissue homogenates. In these studies, we used filtration as a selective process in isolating these organisms. The antigenic load presented by the periodontal microflora, composed of a single type of organism, is equal to that presented

TABLE 4. Serum inhibition of chemotaxis in the Boyden chamber assay[a]

PD patients with either Crohn's disease or ulcerative colitis	Mean chemotactic response as % of control ± SEM	
	EAS	FMLP
Crohn's disease		
1	10.4 ± 2.4	19.2 ± 1.2
2	27.1 ± 2.1	23.5 ± 2.3[b]
3	78.8 ± 6.2	49.7 ± 4.7
4	49.8 ± 3.9	50.1 ± 5.1
5	18.8 ± 1.7	12.3 ± 0.6
6	14.1 ± 0.7	18.3 ± 0.7
Ulcerative colitis		
7	69.4 ± 5.8	57.8 ± 4.3
8	11.9 ± 0.9	22.2 ± 2.1
9	12.7 ± 4.2	20.1 ± 4.5
10	19.2 ± 7.4	12.1 ± 6.7

[a] Normal neutrophils were exposed to 5% serum from patients with IBDs as indicated, and normal serum served as control. Sera from patients with both IBDs markedly inhibited normal neutrophil chemotaxis. For each patient, age- and sex-matched controls were used for each test.

[b] Not statistically significant at $P < 0.05$.

in the terminal ileum and colon. Considering the continuity of the oral cavity and gastrointestinal tract, further investigation into the role of these organisms as potentially infective agents seems warranted.

As is indicated by the clinical measurements, the degree of inflammation of the gingiva of the affected IBD patients seems greater than that of age- and sex-matched periodontal disease patients. This is also reflected in the CF-PGE_2 level of these individuals which is fourfold that of the periodontitis group. It is of note that in adult periodontitis, PGE_2 levels in CF correlate well with active disease episodes (38) and tissue destruction. These data suggest that, on the average, the group with IBD and periodontal disease is exhibiting more inflammation and perhaps more tissue destruction.

The basic immune function of patients with IBD has been reported to be abnormal in isolated cases (28). However, no clear pattern has emerged, and it is difficult to reconcile the many conflicting reports regarding antibody production, complement activation, and immediate and delayed hypersensitivity reactions in these patients. However, there is a clear increase in the frequency of allergy to dietary proteins in IBD patients (43). Anticolon antibodies have been described, first by Broberger and Perlmann in 1959 (6), in both ulcerative colitis and Crohn's disease. However, it seems unlikely that these antibodies play any major role in the pathogenesis of IBD, because there is no correlation of antibody titer with any clinical parameter and anticolon antibodies are found in Crohn's disease patients with lesions only in the ileum.

The main thrust of recent investigations has been the cytotoxic effects of cultured lymphocytes obtained from IBD patients. These killer lymphocytes (non-T and non-B cells) are thought to function in antibody-dependent cell-mediated cytotoxicity. They are cytotoxic for colonic but not ileal epithelial cells and therefore seem to be more relevant to the pathogenesis of ulcerative colitis only.

Immune complexes have been demonstrated in the serum of IBD patients by a variety of techniques (8, 21, 25; W. F. Doe, D. Yang, and P. M. Henson, Gastroenterology (abstr.) p. 1157, 1977) and have been localized in the basement membrane of rectal tissues. Little is known about these immune complexes. The antigen(s) is unknown, and the possibility of a laboratory artifact exists. Moreover, the specificity of the localization of protein in inflamed tissues is questionable. However, depletion of complement in IBD patients and the physical nature of the lesion suggest a major role for immune complex pathology in the pathogenesis of IBD.

Reports of neutrophil function in IBD have been conflicting and often hard to interpret because of differences in methodologies and categorizations of disease groups. Our data demonstrate serum-mediated inhibition of neutrophil chemotaxis in Crohn's disease and in ulcerative colitis. Considering data from other laboratories regarding the role of immune complexes in the pathogenesis of IBD, it is not unexpected to find chemotaxis inhibited, since immune complexes are known to have an inhibitory effect upon neutrophil chemotaxis. Phagocytosis of opsonized *S. aureus* by neutrophils from IBD patients does not differ from the normal.

In the context of bacteria-host interactions, the *Wolinella* isolates had a profound effect on neutrophil chemotaxis in vitro. Similar to results obtained with peripheral blood neutrophils, phagocytosis was normal even in the presence of the *Wolinella* extracts. Based upon previous observations (12, 27, 41), the periodontal manifestations of IBD in these patients can be in part attributed to the decrease in neutrophil function. Moreover, the neutrophil dysfunction may be mediated by an infectious agent. There is precedent in the work of Shurin et al. (44) for the concept of an oral organism affecting peripheral blood neutrophil function; an oral organism, a member of the genus *Capnocytophaga*, was suggested to have a transient suppressive effect on neutrophil chemotaxis, which was reversed after elimination of the organism. Elimination of *Wolinella* organisms from the periodontium of IBD patients has proven difficult because the patients do not respond to conventional periodontal therapy. Studies are under way to attempt to eliminate the organism and to observe the subsequent effect on neutrophil chemotaxis inhibition by serum.

We attempted to provide further insight into the mechanisms of the pathogenesis of the IBDs. The isolation of an organism that by virtue of its numerical density presents an enormous antigenic load to the host and via its interaction with neutrophils inhibits neutrophil chemotaxis sheds new light on the problem of the isolation of infectious agents in IBD. Further work in this area will focus on colonization of the gut by *Wolinella* and *Campylobacter* species and the humoral and cell-mediated host response to these organisms.

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